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Effect of Digoxin on Reproductive Function in Female Wistar Rats

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Abstract

Aim: This study was designed to investigate the effect of digoxin on reproductive function in female Wistar rats.

Methods: Sixteen female rats (120 – 160 g) were used for the estrous cycle and histopathological studies. Digoxin (0.71 mg/kg) was administered orally on daily basis for 21 and 50 days respectively for the estrous cycle and histological studies. Estrous cycle was carried out using the technique of Marcondes *et al*., histologies of the ovaries and uteri were also carried out. Data were analysed using descriptive statistics and student's t-test at p=0.05.

Results: Treatment of rats for 21 days with digoxin (0.71 mg/kg) produced a significant ($p<0.05$) decrease in the estrous phase, but caused insignificant ($p>0.05$) changes in the proestrous, metestrous and diestrous phases of the estrous cycle relative to their respective controls. The histopathological study presented with a moderately congested ovarian medulla, but no visible lesion was seen in the uteri of the rats.

Conclusion: It can therefore be concluded that digoxin probably has anti-fertility effect with a moderate deleterious effect on the ovaries but non-deleterious effect on the uteri at histological level in female Wistar rats.

Keywords: Digoxin, Proestrous, Estrous, Ovaries, Rats.

INTRODUCTION

Digoxin is a cardiac glycoside used to treat various heart conditions. Most frequently it is used for atrial fibrillation, atrial flutter, and heart failure. Digoxin was first isolated in 1930 from the foxglove plant, *Digitalis lanata* [1]. It is on the World Health Organization's List of Essential Medicines, the most effective and safe medicines needed in a health system [2].

Digoxin has been reported to play an important role in iron transport into heart in iron overload state in rats [3]. Its cardiac toxicity effect in newborn and adult rats has been reported [4]. Digoxin long-term administration has been reported to cause up-regulation of the Na^+ K^+-ATPase in most tissues [5]. Its distruptive effect on inflammatory response in experimental *Pneumococcal pneumonia* has been reported [6]. It has been reported to inhibit development of hypoxic pulmonary hypertension in mice [7] and its retinal degeneration induction has been reported to depend on rhodopsin [8]. Its effect on reproductive function in male rats has also been reported [9]. However, due to scanty information from literature on the effect of digoxin on reproductive parameters in female rats, this study therefore aims at investigating the effect of this antiarrhythmic agent on these aforementioned parameters in female rats.

MATERIALS AND METHODS

Experimental Animals

Adult female rats weighing between 120 g – 160 g bred in the Pre-Clinical Animal House of the College of Medicine and Health Sciences, Afe Babalola University were used. They were housed under standard laboratory conditions and had free access to feed and water; they were acclimatized for two weeks to laboratory conditions before the commencement of the experiments. All experiments were carried out in compliance with the recommendations of Afe Babalola University Ethics Committee on guiding principles on care and use of animals.

Drug

Digoxin tablets (Elix-Rox, Ltd.) were bought from Danax Pharmacy, Ibadan, Nigeria.

Digoxin (0.25 mg) was dissolved in 10 ml of distilled water to give a concentration of 0.025 mg/ml.

The dosage of digoxin used in this study was in accordance with that reported by the manufacturer.

Experimental Design

Study on Estrous Cycle

Six matured female rats showing at least three regular 4 – 5 day cycles were used for this study. Vaginal lavages (smears) were examined microscopically every day at a constant interval of 6.30 – 7.30 a.m. for 21 days before and after treatments with the antiarrhythmic drug. The smears were classified into one of the phases of the estrous cycle using the Marcondes technique [10].Vaginal secretion was collected with a plastic pipette filled with 10 µL of normal saline (NaCl 0.9 %) by inserting the tip into the rat's vagina, but not deeply. Vaginal fluid was placed on glass slide. One drop was collected with a clean tip from each rat. Unstained material was observed under a light microscope, without the use of condenser lens, with 10 and 40 x objective lenses. Three types of cells could be recognized: round and nucleated ones are epithelial cells; irregular ones without nucleus are the cornified cells; and the little round ones are the leucocytes. The proportion (preponderance) among them was used for the determination of estrous cycle phases [11, 12]. The duration of the estrous cycle was determined. In this study, the experimental animals also served as the control. The first 21 days served as the control days, while the last 21

days served as the treatment days. Each of the 6 rats for this estrous cycle study received 0.71 mg/kg of digoxin.

Histopathological Study

In another set of experiment, ten matured female rats divided into two equal groups (five animals per group) received the following treatment of the antiarrhythmic agent and control orally per day for fifty days as follows:

Group I rats received 0.5 ml/100 g of distilled water as the control group.

Group II rats received 0.71 mg/kg of digoxin.

On the 51st day, all the rats were sacrificed by an overdose of diethyl ether. The ovaries and uteri were dissected out, cleaned of fat and immediately fixed in Bouin's fluid.

Histological preparation of tissues

After weighing the ovaries and uteri, they were immediately fixed in Bouin's fluid for 12 hours and the Bouin's fixative was washed from the samples with 70 % alcohol. The tissues were then cut in slabs of about 0.5 cm transversely and the tissues were dehydrated by passing through different grades of alcohol: 70 % alcohol for 2 hours, 100 % alcohol for 2 hours, and finally 100 % alcohol for 2 hours. The tissues were then cleared to remove the alcohol, the clearing was done for 6 hours using xylene. The tissues were then infiltrated in molten paraffin wax for 2 hours in an oven at 57oC, thereafter the tissues were embedded. Serial sections were cut using rotary microtone at 5 microns (5µm). The satisfactory ribbons were picked up from a water bath (50 -55oC) with microscope slides that had been coated on one slide with egg albumin as an adhesive and the slides were dried in an oven. Each section was deparaffinized in xylene for 1 minute before immersed in absolute alcohol for 1 minute and later in descending grades of alcohols for about 30 seconds each to hydrate it. The slides were then rinsed in water and immersed in alcoholic solutions of hematoxylin for about 18 minutes. The slides were rinsed in water, and then differentiated in 1 % acid alcohol and then put inside a running tap water to blue and then counterstained in alcoholic eosin for 30 seconds and rinsed in water for a few seconds, before being immersed in 70 %, 90 % and twice in absolute alcohol for 30 seconds each to dehydrate the preparations. The preparations were cleared of alcohol by dripping them in xylene for l minute. Each slide was then cleaned, blotted and mounted with DPX and cover slip, and examined under the microscope. Photomicrographs were taken at x40 and x100 magnifications.

Statistical Analysis

The mean and standard error of mean (S.E.M.) were calculated for all values. Comparison between the control and the treated group was done using student's t-test. Differences were considered statistically significant at $p<0.05$.

RESULTS

Treatment of rats for 21 days with digoxin (0.71 mg/kg) produced a significant ($p<0.05$) decrease in the estrous phase, but caused insignificant ($p>0.05$) changes in the proestrous, metestrous and diestrous phases of the estrous cycle relative to their respective controls (Fig. 1).

Treatment of rats with digoxin (0.71 mg/kg) for 50 days produced moderately congested ovarian medulla, which is contrary to what was observed in the control rats (Plates 1 and 2).

Treatment of rats with digoxin (0.71 mg/kg) for 50 days produced no visible lesion on the uteri, which is similar to what was observed in the control rats (Plates 3 and 4).

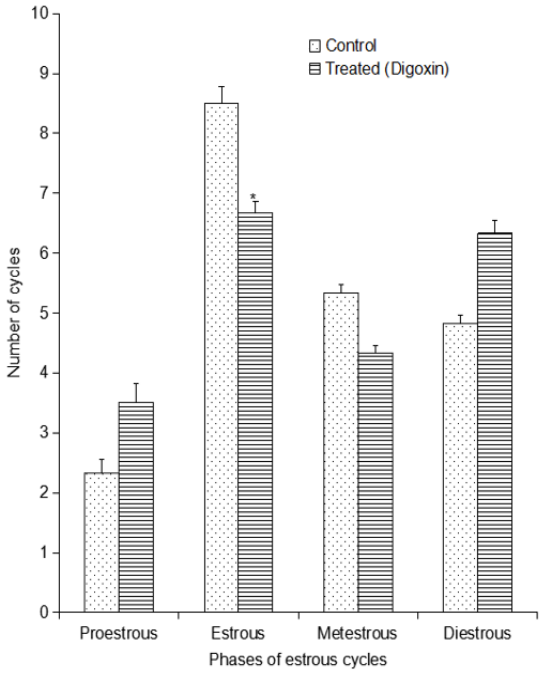

Fig. 1: Effect of 21 days treatment with digoxin on estrous cycle (n = 6, *p<0.05)

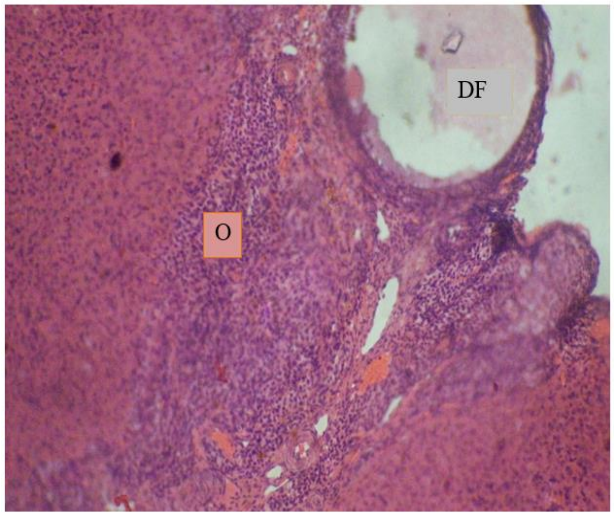

Plate 1: Effect of 0.5 ml distilled water (control) on the ovary at x100.

Photomicrograph showing a normal ovary (O) with a developing follicle (DF).

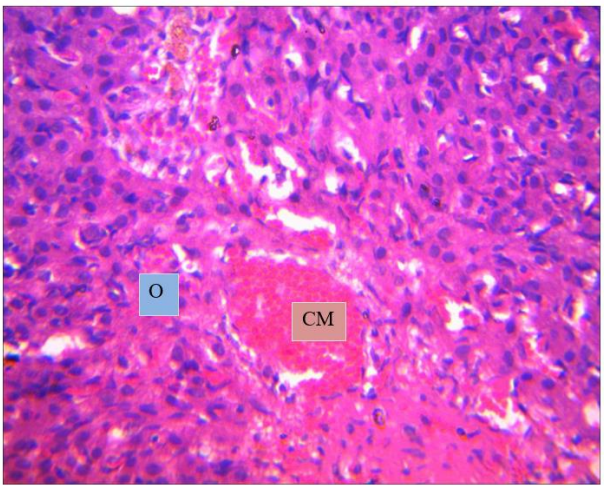

Plate 2: Effect of digoxin (0.71 mg/kg) on the ovary at x100
Photomicrograph showing an ovary (O) with moderately congested medulla (CM).

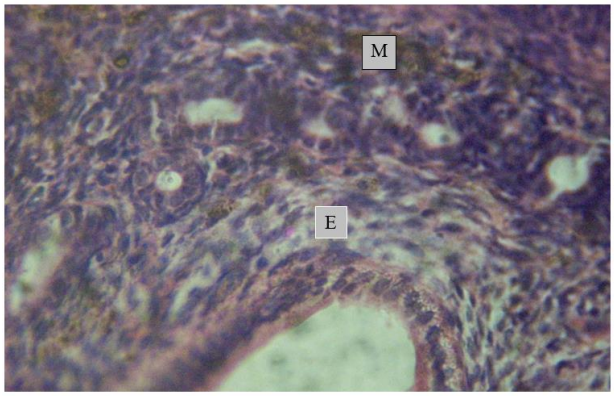

Plate 3: Effect of 0.5 ml distilled water (control) on the uterus at x100.
Photomicrograph showing normal endometria (E) and myometrium (M).

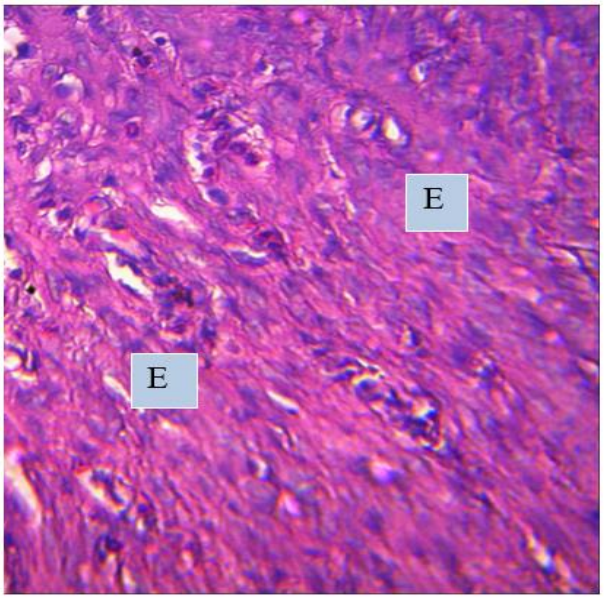

Plate 4: Effect of digoxin (0.71 mg/kg) on the uterus at x100
Photomicrograph showing endometrial (E) layer with no pathologic lesion seen.

DISCUSSION

The estrous cycle study revealed that digoxin caused a significant change in the duration of a phase of the estrous cycle. Contrary report was given by [13] in *Portulaca oleracea* extracts treated rats. This suggests that the antiarrhythmic drug caused imbalances of the ovarian and extraovarian hormones, since it has been reported that imbalance in these hormones lead to irregularity in the ovarian functions and duration of the estrous cycle [14].

Treatment of rats with digoxin caused significant decrease in estrous phase of the estrous cycle which suggests the non-availability of matured Graafian follicles and would not lead to ovulation. Contrary result was reported by [15] in alcohol treated rats.

The ovarian photomicrographs of the digoxin treated rats presented with moderately congested ovarian medulla which could be due to deep venous thrombosis. This is similar to the result obtained by [16] in Sumithion treated rats.

The uterine photomicrographs of the digoxin treated rats revealed no pathologic lesion which probably indicates the non-toxic effect of the drug on the uteri at histological level. Similar results were reported by [17] in *Allium sativum* extract treated rats.

It can therefore be concluded that digoxin probably has anti-fertility effect with moderate deleterious effects on the ovaries but non-deleterious effects on the uteri at histological level in female Wistar rats. However, the effect of digoxin on human reproductive function is unknown; nevertheless, considering these findings in animal model, it is recommended that women should be cautious about taking this antiarrhythmic agent because of its likely anti-fertility and ovarian deleterious effect.

Conflict of Interest

We vehemently declare that there is no conflict of interests in this research work.

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